Evaluation of Two Commercially Available ELISA Kits for Detection of Salmonellae in Swine Lymph Nodes and Cecal and Fecal Contents

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Abstract

We conducted a survey to determine the prevalence of salmonellae in pigs in an integrated swine operation. Isolation of salmonellae from lymph nodes, cecal contents, and fecal samples was by routine microbiological culture techniques. Simultaneously, and using the same samples, we evaluated two commercially available ELISA-based *Salmonella* test systems (Neogen Reveal[R] and EiaFoss™) for detection of salmonellae and compared these results to our culture results. The advantages of the Neogen test were that it was simple to use and had up to an 87% agreement with culture. The disadvantages of the Neogen test were that it was expensive, it was not suitable for cecal or fecal samples (cross-reacted with *Citrobacter* spp.), it had a high rate of false negatives, and positive endpoints were subjective and difficult to read. The advantages of the Foss test were that it had up to a 93% agreement with culture results, it had low rates of false negatives and false positives, it was applicable for cecal and fecal samples, and it reduced time and labor compared to culture procedures. The disadvantages of the Foss test were that it required purchase of an expensive auto analyzer and not all of the 3 protocols recommended by the manufacturer were equally effective in recovery of salmonellae. We concluded that under our study conditions, the Neogen test was not acceptable, but that the Foss system was suitable for detection of salmonellae from field samples.

Introduction

Losses to the U.S. swine industry from *Salmonella* species are estimated at >$100 million annually. *Salmonella*-contaminated meat, including pork, is an important source of infection for human salmonellosis. Due to public health concerns, there has been an increased effort to survey the incidence of on-farm salmonellae in swine production (2). Additionally, pathogen reduction control programs, such as the Hazard Analysis of Control Points program, have been developed as a method to evaluate and reduce contamination of pathogens such as salmonellae in swine and pork. With this increased emphasis on testing of commodities, it would be advantageous to have access to methods that reduce the time necessary to know the microbiological status of products.

Standard culture techniques for *Salmonella* may take 5-7 days for a positive or negative determination. Numerous ELISA-based tests have been proposed for rapid screening of food products and environmental samples (1,5,6). The purpose of this study was to evaluate 2 commercial ELISA rapid tests, licensed for food samples, to detect salmonellae from field samples utilizing protocols slightly modified from those recommended by the manufacturer. Field samples consisted of swine lymph nodes and cecal contents from slaughter and rectal swabs or fecal samples from live pigs. We then compared these results to standard culture techniques. The rapid tests were Reveal,[R] a sandwiched antibody ELISA from Neogen Corp. that utilizes a cassette with a micro well, and EiaFoss,™ from Foss Electric that incorporates an ELISA bound to electromagnetic beads with detection by an auto analyzer.

Materials and Methods

Culture procedures—Each swab or sample (0.2 to 5.0 g) was first enriched in tetrathionate broth, further enriched in Rappaport-Vassiliadis (RV) broth, and plated onto brilliant green agar medium (BGA) containing novobiocin (25 µg/ml). Enrichment and differentiation were accomplished at 37° C during 24 h incubation. Salmonellae-like suspect colonies were tested by agglutination with *Salmonella* O Antiserum Poly A-1 and Vi.

Reveal procedures—The manufacturer recommends that 25g of food product be added to 200 ml of proprietary pre-enrichment broth, incubated at 37° for 4 h, enriched with 200 µl of RV broth, incubated at 43° C for 16 h, and 3 drops (approximately 100 ml) placed onto the test cassette. A positive test is indicated by development of a colored line on the cassette. We introduced 3 variations of this protocol by reducing the amount of inoculum along with reductions in the amount of enrichment broth. They were: 5 g of lymph node or 1 g cecal or fecal material added to 200 ml, 80 ml, or 40 ml of pre-enrichment and enriched with 200 ml, 80 ml, or 40 ml of RV (incubation times and temperatures remained as recommended).

FossEia procedures—Foss has 3 recommended protocols (raw foods, processed foods, and feed) for salmonellae recovery. The raw food protocol (24 h) recommends that 25 g of product be inoculated into 225 ml of primary enrichment, incubated at 41° C for 16 to 24 h, transferred to a post-enrichment, incubated at 42° C for 3 h, and then samples be loaded and run by the auto analyzer. The processed food protocol (24 h) recommends that 25 g of product be inoculated into 400 ml of primary enrichment and incubated at 37° C for 4 h, further incubated at 41° C for 15 to 20 h; transferred to a post-enrichment and incubated at 42° C for 3 h; and then samples be loaded and run by the auto analyzer. The feed protocol (48 h) recommends that a 25 g sample be inoculated into 400 ml of primary enrichment and incubated at 37° C for 4 h, further incubated at 41° C for 15 to 24 h; transferred to a selective enrichment and incubated at 42° C for 18 to 26 h; transferred to a post-enrichment and incubated at 41° C for 2.5 h; and then analysis be performed by the auto analyzer. In our modified tests, regardless of the protocol used, we inoculated 5 g of lymph node or 1 g cecal or fecal contents into 20 ml of the primary enrichment and followed the recommended protocol from that point. In the case of rectal swabs, we inoculated swabs into 10 ml of primary enrichment and proceeded as recommended.

Serial Dilutions—In order to determine the concentration of salmonellae necessary to induce a positive response, serial dilutions (10^{-1} to 10^{-9}) were made from pure cultures of *S. typhimurium* and *S. schwarzengrund*, and tested by the two ELISA methods. Each of the dilutions was then streaked onto agar plates and colony-forming units (cfu) determined. Serial dilutions (10^{-4} to 10^{-9}) were also made of *Citrobacter freundii* and this organism tested by the two ELISA techniques.

Results and Discussion

Reveal—Salmonellae detection from swine lymph nodes and cecal contents varied according to the type of sample and on our modifications of the recommended procedure (Table 1). Our data show that when the volume of enrichment was varied, the results from the Reveal test for lymph nodes were similar, but the use of 200 ml of enrichment gave slightly better detection than either 80 ml or 40 ml. When compared to culture results, the lymph node test results were considered undesirable due to the percentage (14% to 21%) of salmonellae that went undetected, the high rate of suspicious reactions (10%, 4.2%, and 14.9% for the 200 ml, 80 ml, and 40 ml protocols, respectively), and the high percentage of false negative responses. We removed all suspicious reactions from our calculations since we could not consider them in either the positive or negative categories. Unacceptably high levels of false positive, false negative, and suspicious reactions have been reported as disadvantages to the Reveal test when evaluating field samples from poultry houses (5). We chose to reduce the amount of tissue in this study because it is difficult to collect 25 g of ileocolic lymph node tissue from market age swine at slaughter; however, 5 g is feasible. We hypothesized that if we reduced the amount of tissue, we could also reduce the volume of enrichment and this would make the test more practical for field use. Unfortunately, we did not compare our modification to the manufacturer's recommendations (i.e., 25 g tissue and 400 ml enrichment), so we do not know what level of detection we would have achieved with those procedures. However, when the manufacturer's recommendations were followed, similar results to ours have been reported (5). In our opinion, drawbacks to the Neogen product are the large volume of enrichment needed and the plastic bags that are used for the enrichment process. The bags are bulky, do not remain upright on counter tops, and are prone to leakage. When we tested samples of cecal contents with the Reveal test, there were 100% positive responses. Suspecting that the antibody might cross-react with *Citrobacter* spp., we tested serial dilutions of *C. freundii* and found that concentrations of 10^4 cfu and above tested positive. Therefore, we concluded that the Reveal test kit was not suitable for rectal swabs or cecal or fecal samples. The manufacturer states that the Reveal test can detect *S. typhimurium* and *S. schwarzengrund* at concentrations of 10^4 cfu (4). However, when we tested known concentrations of these organisms, the Reveal test did not react positively until 10^6 cfu or greater were used.

EiaFoss—As in the Neogen test, variation in results appeared to be due to the type of protocol used (Table 1). When comparing the 3 protocols, the feed protocol had the highest percentages of salmonellae detection and this is probably due to the extra 24 h of enrichment and incubation. For lymph nodes, the Foss and Neogen products overall had similar detection rates. However, when the feed protocol was used on cecal or fecal samples or rectal swabs, the results from the Foss test compared very favorably to standard culture methods. Results similar to ours have been reported for the Foss test when used for cecal and fecal samples (6). In that study, the sensitivity (0.98) and the specificity (0.99) for salmonellae were high and the false positive rates were low. In the present study, the feed protocol produced low rates of false positive and false negative responses and no suspicious responses for lymph node or cecal/fecal samples. Although Foss states that their test will detect *S. typhimurium* and *S. schwarzengrund* at 10^5 cfu (3), when we tested known concentrations of these organisms, positive responses were not achieved until 10^6 cfu or greater were used. The Foss test gave negative responses to all concentrations (up to 10^9 cfu) of *C. freundii* used.

Summary and Conclusions

The advantages of the Reveal test was that it was simple to use, it did not require operator training or sophisticated laboratory equipment, all necessary materials were supplied

in a kit by the manufacturer, results were available within 24 h, and it had up to an 87% agreement with standard culture for detection of salmonellae. The disadvantages were that the bags supplied for the enrichment broth were difficult to work with, the rate of false negative reactions was high, the endpoints for reading the test were subjective and difficult to determine, the cost for each test was $12 to $15, the antibody cross-reacted with *Citrobacter spp.* and was not suitable for use on cecal or fecal samples, and the detection limits were at 10^6 cfu or greater. The advantages of the EiaFoss test was that it had up to a 93% agreement with standard culture, it had a low percentage of false positive or false negative reactions, it had no suspicious reactions, results were available within 24 to 48 h, it could be used for cecal or fecal samples, and it reduced labor costs compared to culture. The disadvantages were that it was expensive (auto analyzer costs $35,000), not all the protocols were equally effective in salmonellae detection, detection limits were at 10^6 cfu or greater, and the auto analyzer required some operator training.

Under the conditions of our study, we concluded that the Neogen Reveal test was not suitable, but that the EiaFoss test was acceptable for detection of salmonellae from field samples.

Table 1 Comparison of 2 ELISA tests with culture results for detection of salmonellae

Sample type or protocol	Agreement with culture (%)	False negative (%)	False positive (%)
Neogen Reveal			
Lymph node (N=244)			
200 ml	86.7	11.1	2.2
80 ml	82.6	12.2	5.2
40 ml	79.4	19.0	1.6
Cecal content (N=10)			
200 ml	0	0	100
EiaFoss			
Lymph node (N=259)			
Raw food	72.0	21.5	6.2
Processed food	60.5	36.8	2.6
Feed	84.6	3.8	1.2
Cecal/fecal (N=582)			
Raw food	76.7	15.0	8.3
Feed	92.5	4.4	3.1

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